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Grain yield, efficiency and the allocation of foliar N applied to soybean canopies

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ABSTRACT: Soybean [*Glycine max* (L.) Merr.] grain yield is closely associated with the level of optimal nitrogen (N) supply, especially during the reproductive stages. Foliar fertilization with low rates of N have been considered as a strategy for furnishing additional N and enhancing grain yields. Field studies using ^{15}N tracer were conducted over two growing seasons to investigate the impact of foliar N fertilization on grain yield, plant N content, the amount of N derived from fertilizer (NDFF) and N recovery efficiency (NRE). Four foliar N rates (0, 1300, 2600 and 3900 g ha^{-1}) were supplied by two equal split applications at the R1 and R3 stages. Foliar N fertilization of soybean canopies did not affect grain yield, grain N content, shoot N content nor plant N content. Total NDFF was increased from 0.7 to 2.0 kg ha^{-1} across the N rates. Nonetheless, NRE was unaffected by foliar N fertilization, which averaged 53 %. Soybean plants allocated the same amount of N fertilizer to both grains and shoots. No significant effects of low rate foliar N fertilization were registered on soybean grain yield nor plant N content, despite considerable N fertilizer recovery by plant organs.

Keywords: *Glycine max* (L.) Merr, foliar N fertilization, ^{15}N tracer, foliar application, nitrogen recovery efficiency

Introduction

Foliar nitrogen (N) fertilization is an increasingly common practice in commercial grain soybean [*Glycine max* (L.) Merr.] production. Soybean N fixation will necessarily reflect variations in soil N supplying power, and although soybeans can fix appreciable amounts of N ~200 kg ha^{-1} (Unkovich and Pate, 2000; Alves et al., 2003; Herridge et al., 2008), recent studies document that N limits grain yield (Wilson et al., 2014; Felipe et al., 2016). The application of low amounts of N as a foliar spray is a pathway for additional N, especially during the pod-filling stage when N demand is high (Gaspar et al., 2017). In addition, compatibility with pesticides commonly applied reduces costs and more foliar N applications may be performed.

Previous studies have shown grain yield increases resulting from foliar N fertilization (Vasilas et al., 1980; Blandino and Reyneri, 2009; Ranđelović et al., 2009; Jyothi et al., 2013; Khan et al., 2013). It has been suggested that foliar N application may provide other advantages as well. Using foliar N application during the pod-filling, Ikeda et al. (1991) reported increased photosynthetic productivity in leaves and the export of photosynthates to the nodules for an extended length of time. In studies investigating foliar application effects on grain N concentrations, such concentrations increased between 0.2 % and 2.4 % (Ruske et al., 2003; Blandino and Reyneri, 2009; Mandić et al., 2015).

In contrast, a lack of grain yield effect has also been observed in many field studies. Excessive foliar damage may limit grain yield responses when high rates of N are applied under leaves (Phillips and Mullins, 2004). However, two reviews performed by Gooding and Davies (1992) and Fageria et al. (2009) showed that grain yield responses are also closely correlated with foliar application timing. The same authors observed that grain yield increases were most commonly obtained through foliar application during the reproductive stages and with negligible foliage burning.

While these studies provide valuable information about foliar N fertilization, only a few studies have investigated foliar fertilizer recovery efficiency in soybean crops. Moreover, previous studies have involved higher rates of foliar N fertilizer than are used in Brazilian soybean production to supply N while avoiding leaf damage. Knowledge of foliar N recovery efficiency (NRE) and its allocation in soybean crops could help to better implement foliar N application strategies and to better understand the effects on grain yield. Therefore, using ^{15}N tracer methods, the main goal of this study was to investigate the effects of foliar N fertilization on grain yield and NRE.

Materials and Methods

Site description

The field experiment was performed in the state of São Paulo (49°15′08″ W, 23°34′53.5″ S, 545 m asl) over two growing seasons in 2012-2013 (Year 1) and 2013-2014 (Year 2). Located in the southwest of Brazil, this experimental area has a subtropical humid climate (Köppen, 1936) with low seasonal precipitation variation. The rainfall and mean temperature for crop seasons are shown in Figures 1A and B. Based on the USDA (1999) classification system the soil is classified as a Typic Hap-

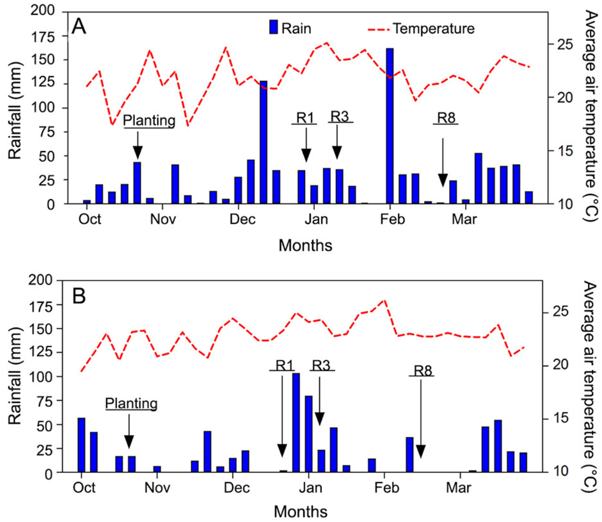

Figure 1 – Rainfall and average air temperature during the soybean growing season in 2012-13 (A) and 2013-14 (B). Planting and growth stages are specified in accordance with Fehr and Caviness (1977).

ludalf with 657 g kg^{-1} clay, 253 g kg^{-1} silt and 190 g kg^{-1} sand. Before planting, the soil chemical properties were measured at a 0-20 cm depth, and provided the following results: pH of 5.5 ($CaCl_2$), 40 g of organic matter dm^{-3}, 2.2 g of N kg^{-1}, P (resin extractable) of 19 mg kg^{-1}, exchangeable K of 0.76_{cmolc} kg^{-1} and base saturation of 70 %.

The experiment was conducted using a randomized block design with four replicates. Treatments consisted of four foliar N rates (0, 1300, 2600 and 3900 g ha^{-1}) applied as urea through two equal split applications at R1 and R3 stages (Fehr and Caviness, 1977). No foliage burning was noted across the applications. Each plot consisted of 10 m-long rows spaced 0.45 m apart. The Nideira 5909 RR variety was planted at 226,000 and 247,000 seeds ha^{-1} for years 1 and 2, respectively. Phosphorus and potassium were applied in the furrow during planting as triple superphosphate (86 kg ha^{-1} P_2O_5) and KCl (65 kg ha^{-1} K_2O).

N internal efficiency (NIE) indicates how efficiently soybean grains produce relative to the N accumulated by the aboveground biomass (kg grain produced/kg aboveground biomass N content) (Fageria, 2014).

Foliar N fertilizer was applied using a manual backpack sprayer pressurized with CO_2 and equipped with a flat spray tip applying a 200 L ha^{-1} spray volume. The soybean canopy was situated 0.5 m below the spray tip.

^{15}N methods

Each treatment plot, a microplot with four 1 m rows and a total area of 1.8 m^2 received labeled fertilizer spread containing 2.53 atom % ^{15}N, according to the rates and stages described earlier. The remaining plants in the plot were treated with unlabeled fertilizer.

Labeled samples were taken at the R7 stage (Fehr and Caviness, 1977) to evaluate the total N and the N derived from fertilizer (NDFF) amounts in the soybean biomass above ground. Four plants were collected from each of the two rows in the middle of the microplots and divided into grain and shoot categories (petiole, leaf, steam and pod). This early sample aimed to avoid leaf drop and increase the non-recovery of ^{15}N (NRN). Grain yield was obtained using unlabeled samples taken at physiological maturity and by adjusting the moisture level to 130 g kg^{-1}. Approximately 100 plants were collected within 3 m of the three central rows of each plot.

All samples were dried in a forced air circulation laboratory oven at 65 °C, weighed and carefully ground into fine powder using a Wiley mill. The $^{15}N/^{14}N$ ratios as well as the total N concentration in plant samples were determined in an automated mass spectrometer coupled to an ANCA-GSL N analyzer (Sercon Co., UK). The term N recovery efficiency (NRE) was used to indicate the percent of N fertilizer recovery by the whole plant. The NDFF was used to indicate the amount of N fertilizer recovery in compartments of the whole plant, expressed in kg ha^{-1}. NDFF values were obtained using the following equation.

$$\text{NDFF (kg ha}^{-1}) = \left[\frac{\alpha-\beta}{\gamma-\beta}\right] . \textit{total N} \quad (1)$$

where: NDFF is the amount of *N* derived from the fertilizer (kg ha^{-1}), α the abundance of ^{15}N atoms in the sample (%), β the natural abundance of ^{15}N atoms, γ the abundance of ^{15}N atoms in the fertilizer (2.53 % atoms), and total N the total N ($^{15}N + ^{14}N$) contained in the sample (kg ha^{-1}).

$$\text{NRE (\%)} = \left[\frac{\text{Total NDFF}}{\text{N rate}}\right] . \textit{total N} \quad (2)$$

where: NRE is the percentage of ^{15}N recovered from the whole soybean plant, Total NDFF the amount of ^{15}N recovered from whole plant (kg ha^{-1}), and the fertilizer *N* rate the rate of enriched fertilizer applied (kg ha^{-1}).

Statistical analyses

All data were analyzed using the statistical software program (SAS v. 9.2, 2009). Before analysis, the observations from the response variables were tested for homoscedasticity using the Box-Cox test (Box and Cox, 1964). F-tests were performed at a 5 % probability to identify the effects and their interactions. If the null hypothesis was rejected, the mean was compared via Fisher's least significant difference (LSD) test at $p \leq 0.05$ probability, and regressions were fitted to the rates when necessary.

Results

Foliar N fertilization had no effect on grain yield (Table 1) and yield components such as the number of nodes, pods, stems or seed weight (data not shown).

Nonetheless, we registered differences between grain years. Grain yield means were 3.7 and 4.2 Mg ha^{-1} for years 1 and 2, respectively. Plant N content and NIE had similar responses to foliar N application, and while no difference was registered across rates, differences between years were significant for grain and plant N content. Grain and plant N content ranged between 185-197 kg ha^{-1} and 254-281 kg ha^{-1}, respectively, throughout the two years of the study. Average shoot N content was 79 kg ha^{-1}.

As might be expected, high rates of foliar N resulted in greater grain NDFF. There were statistical differences between years (Year 1 > Year 2), but no interaction between year and rate (Table 2). A positive linear regression between grain NDFF and rates was fitted (Figure 2A).

There was no difference in NDFF between years and their interaction with rates. Nevertheless, the shoot NDFF was significantly different across the rates and ranged from 0.32 to 0.83 kg ha^{-1} (Figure 2B).

As noted for grains and shoots, total NDFF was significantly affected by rates. Despite the year difference, there was no interaction effect between year and rate. Our regression analysis showed that total NDFF increased ~0.5 g N kg^{-1} when N fertilizer was applied (Figure 2C).

There was no difference between the rates of foliar N applied for NRE. Even though the years differed, the interaction between year and rate was not significantly affected (Table 2 and Figure 2D). On average, NRE was 53 % for foliar N applications performed on soybean canopies.

N fertilizer distribution is shown in Figures 3A and B. Except for the higher rate in year 1, the same amounts of N fertilizer were allocated in the grains and shoots (p > 0.05). Overall, shoot recovery ranged from 18 % to 27 % and grain recovery ranged from 21 % to 42 %.

Table 1 – Grain yield, N internal efficiency (NIE), and N content for grain, shoot and plant for different years and rates.

Treatment Rate	Grain yield	NIE	Grain N content	Shoot N content	Plant N content
g ha^{-1}	Mg ha^{-1}	kg ha^{-1}			
0	3.98	15.9	185	69	254
1300	3.88	13.6	196	85	281
2600	4.00	15.0	192	86	279
3900	4.07	14.2	197	76	273
			Years		
Year 1	3.74 B	14.9	174 B	80	254 B
Year 2	4.22 A	15.0	211 A	78	289 A
			Average		
	3.98	15.0	192	79	271
			ANOVA Pr > F		
Year (Y)	*	ns	*	ns	*
Rate (R)	ns	ns	ns	ns	ns
Y*R	ns	ns	ns	ns	ns
CV %	8.81	5.94	18.1	20.3	16.4

*Significance at $p \leq 0.05$; ns = not significant. Within lines, means followed by different letters are significantly different.

Table 2 – Grain, shoot and total N derived from fertilizer (NDFF) and N recovery efficiency (NRE) for different years and rates.

Treatment Rate	NDFF			NRE
	Grain	Shoot	Plant	
g ha^{-1}	kg ha^{-1}			%
1300	0.35	0.32	0.68	52.0
2600	0.76	0.65	1.42	54.3
3900	1.21	0.83	2.04	52.3
		Years		
Year 1	0.99 A	0.64	1.63 A	60.8 A
Year 2	0.57 B	0.56	1.13 B	44.9 B
		Average		
	0.78	0.60	1.38	52.9
		ANOVA Pr > F		
Year (Y)	***	ns	*	*
Rate (R)	***	***	***	ns
Y*R	ns	ns	ns	ns
CV %	16.7	12.3	13.8	10.5

*Significance at $p \leq 0.05$; ***Significance at $p \leq 0.001$; ns = not significant. Within lines, means followed by different letters are significantly different.

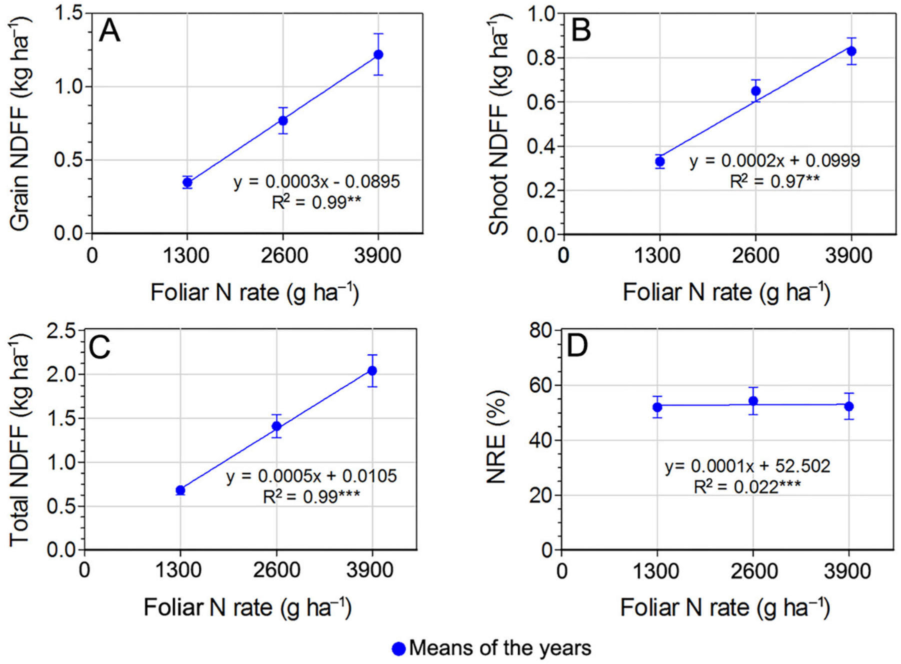

Figure 2 – Foliar N rates effects on grain (A), shoot (B), total NDFF (C) and NRE (D). N derived from fertilizer, NDFF and N recovery efficiency, NRE. **Significance at $p \leq 0.01$; ***Significance at $p \leq 0.001$.

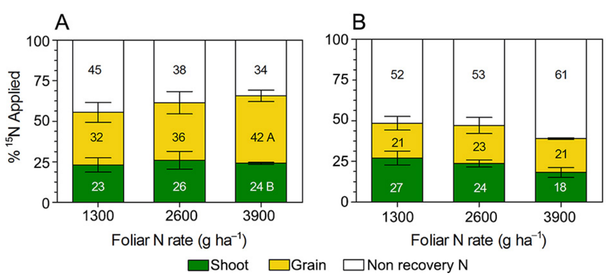

Figure 3 – Allocation of N fertilizer sprayed under soybean leaves. Year 1 (A) and Year 2 (B). Uppercase letters indicate significant difference between organs for the same foliar N rate.

Discussion

Differences between years for grain yield may be a combination of a slight increase (~8 %) of planting rate in year 2 (De Bruin and Pedersen, 2008; Cox and Cherney, 2011), accompanied by better growing conditions (Figure 1A and B). However, N provided by low rates of foliar applications showed no differences in soybean grain yield. Previously reported in the literature, Troedson et al. (1989) and Haq and Mallarino (2000) showed that foliar N fertilization effects on grain yield were usually insignificant. Recently, despite high grain yields (> 4.2 Mg ha^{-1}), Saturno et al. (2017) and Moreira et al. (2017) have found similar results to those in this study using foliar N rates ranging from 5-20 kg ha^{-1} and 5-10 kg ha^{-1}, respectively. For the same studies, only one out of five year sites had significant, but small, grain yield gains (~0.14 Mg ha^{-1}).

Our results agree with the lack of effects of foliar N fertilization on grain yield previously found in literature, but also showed that the lack of effect on grain yield is not linked to low efficiency of fertilizer application since approximately 53 % of the foliar N fertilizer was recovered. Rather, the implication is that low rates of foliar N were of no benefit to the soybean crop, either for directly supplying N or indirectly increasing green leaf area index.

For soybeans, NIE values ranging from 6.4 to 18.8 and with a mean of 12.7 have been reported (Salvagiotti et al., 2008 and references cited therein). Mid to upper NIE values indicate N is the main limiting factor for grain yield. Thus, our results reflect higher NIE (~15) when compared with historical means, demonstrating that the low rates of foliar N might not be enough to support N demand. However, it is not clear how much exogenous N would be the optimal to achieve greater grain yields.

While there was an appreciable increase in NDFF (amount of fertilizer recovery) for all rates, the NRE (percent of fertilizer recovery) was similar, with an average value of 53 % (Figure 2A, B and C). The NRE was comparable to that previously reported by Afza et al. (1987), which was 43-67 % of the total foliar N applied. In a recent study investigating the effects of low rates of foliar N fertilization on soybeans (Pierozan Junior et al., 2015), the authors show a mean NRE of 64 % that was not affected by rates ranging from 650 to 1950 g N ha^{-1}. Thus, foliar N fertilization was an efficient pathway to supply N in soybean plants, despite its lack of effect on grain yield.

Generally, our results showed that foliar N fertilization had little to no effect on ^{15}N partitioning. The lack of differences between grains and shoots could be attributed to N investment in the leaf blends and their photosynthesis rates (Makino et al., 1997). When the N was applied between the R1 and R3 growth stages, the grain sink capacity remained low to medium, but rubisco and light harvesting proteins had a high N demand. Accordingly, Vasilas et al. (1980) reported up to 94 % ^{15}N allocation in the grain when spraying foliar N as urea at the R5-R7 growth stages.

Conclusions

The NRE had no effect across all the foliar N rates and 53 % of N sprayed under leaves was recovered. Overall, grains and shoots allocated N from fertilizer equally well and recovered 29 % and 24 %, respectively. Despite the considerable recovery of N supplied to soybean plants, low rates of N sprayed in the earlier pod-filling stage was not an effective method for enhancing grain yield and dynamic N uptake. The foliar N fertilization effect is not fully understood and is worth further study, especially during pod-filling.

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Authors' Contributions

Conceptualization: Maciel de Oliveira, S.; Almeida R.E.M; Pierozan Junior, C. Data acquisition: Maciel de Oliveira, S.; Almeida R.E.M; Pierozan Junior. C.; Lago, B.C. Data analysis: Maciel de Oliveira, S. Design of Methodology: Maciel de Oliveira, S.; Almeida R.E.M; Pierozan Junior. C.; Lago, B.C.; Trivelin; P.C.O. Software development: Maciel de Oliveira, S. Writing and editing: Maciel de Oliveira, S.; Almeida R.E.M; Pierozan Junior, C.; Favarin, J.L.

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